REPLACED BY ART 34 AMDT

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

4

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
80050/WO		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/01796	02/03/2000	11/03/1999
International Patent Classification (IPC) or national classification and IPC C12N1/14		
Applicant SOCIETE DES PRODUITS NESTLE S.A. et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 1 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
23/08/2000	18.05.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Halle, F Telephone No. +49 89 2399 8537

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/01796

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-16	as originally filed				

Claims, No.:

1-8	as received on	04/05/2001	with letter of	03/05/2001	

Drawings, No.:

1	as originally filed				

Sequence listing part of the description, pages:

1-4, as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☒ contained in the international application in written form.
   - ☒ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:
   see separate sheet

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-8
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-8
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-8
	No:	Claims	

2. Citations and explanations
   see separate sheet

Point I, item 6 (Additional observations)

The application comprises sequence listing sheets numbered 1 to 4.

Point V

1. In this report, it is referred to the following documents:

   D1: Mol. Cell. Biol. 11, 1991, p. 5701-5709 (cited in the application)
   D2: Fungal Gen. and Biol. 22, 1997, pages 28-38
   D3: FEMS Microb. Lett. 151, 1997, p. 103-114
   D4: Microbiology 143, 1997, p. 2991-2998

2. In many microorganisms, a carbon catabolite repression i.e. the repression of proteolytic enzymes which can use less favoured carbon sources occurs when more readily utilized carbon is present in the medium. The creA gene product is known to be responsible for the repression of the synthesis of proteolytic enzymes (in the presence of carbon sources), whereas the areA gene product is known to be responsible for the stimulation of their synthesis.

   In order to increase the proteolytic degradation in the presence of carbon sources, the present invention proposes certain Koji molds having their proteolytic activity not repressed by carbon sources. Said activity not repressed may be due to the altered function of the creA gene or the overexpression of the areA gene.

3. Having regard to the cited prior art, the subject-matter of claim 1-8 appears to be novel and to involve an inventive step.
   The document D1 refers to the assay for the construction of an Aspergillus strain containing a deletion of the entire creA gene. D1, therefore, may be considered as a relevant prior art document. However, it is agreed with the Applicant, that according to the results presented in D1, the creA mutation was unobtainable in a pure haploid condition and that therefore D1 cannot be considered as disclosing a strain wherein the creA-gene is functional. Since claim 1 as presently defined refers to a creA gene mutation and to a non functional corresponding gene product, the subject matter of claim 1 and the related claims 2-8 cannot be

considered as anticipated or rendered obvious by D1.

4. Having regard to the prior art D2-D4, the subject-matter of claims 1-8 appears to be novel and to involve an inventive step. Indeed, although microorganisms with repressed proteolytic activity are known, a Koji mold having such an activity has not been disclosed.

5. Certain documents cited above are not mentioned in the description, cf. Rule 5.1(a)(ii) PCT.

Claims

1. A koji mold belonging to the genus Aspergillus, Rhizopus, Mucor or Penicillium, the proteolytic acitivity of which is not carbon repressed.

2. A koji mold according to claim 1, wherein the creA gene does not exert its inherent function.

3. A koji mold according to claim 2, wherein the creA gene is not transcribed to a mRNA capable to be translated to a functional polypeptide.

4. A koji mold according to any of the claims 1 to 3, wherein the creA gene has been mutated such that the gene product thereof is essentially non functional.

5. A koji mold according to claim 1, wherein the creA gene has been deleted.

6. A koji mold according to claim 1, which is Aspergillus oryzae I-2165 (NF14)

7. A koji mold according to claim 1 to 5, wherein the areA gene or a functional derivative thereof is overexpressed.

8. A method of producing proteolytic enzymes, comprising cultivating a koji mold according to claims 1-7 in a suitable growth medium in the presence of a carbon source under conditions that the mold expresses proteolytic enzymes, and optionally isolating the enzymes in the form of a concentrate.

9. Use of the koji mold according to claim 1-7 for the hydrolysis of protein-containing materials.

10. Use according to claim 8, in combination with an enzyme and/or a microorganism providing a prolidase activity.